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DMBA induced mammary tumor study of Phyto-fabricated silver nanoparticles from *Russelia Equisetiformis* flower extract in rat

Yashraj Yadav

Faculty of Pharmacy, Oriental University Indore [MP]

*Corresponding author email: yashrajyadav@gmail.com

Deshraj Chumbhale

Faculty of Pharmacy, Oriental University Indore [MP]

Abstract--The mammary tumor is the world's most frequent ailment, and it is substantially more common in women. There are several forms of treatment and care available in the globe for tumors, the most common of which being chemotherapy, radiation therapy, and surgery with lots of harmful responses. Cancer has been treated since ancient times by herbs with various herbs and metals. synthesized AgNPs-REEE were characterized by Analytical methods such as dynamic light scattering (DLS), Zeta potential, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDX) and Dynamic Light Scattering (DLS). All of the animals in this study had tumors caused by DMBA Apart from the vehicle control group, and they were divided into different groups such as only DMBA(7,12-dimethylbenz[a]anthracene) induced group, treatment group of ethanolic extract of *Russelia equisetiformis* (REEE) flower and silver nanoparticles of ethanolic extract of *Russelia equisetiformis* (REEE) flower and silver nanoparticles of ethanolic extract (AgNPs -REEE). the dose were decide by acute toxicity study. After compaction treatment time all animal were sacrifices and evaluate parameter like tumor weight, Tumor volume, biochemical parameter, hormonal assay and histopathology of breast tumor. It is observed that the size distribution of AgNPs ranges from 50 to 950 nm. The average size of REE-AgNPs synthesized from 283.6 nm and Polydispersity Index is 0.483. The zeta potential was found to be -12.34. mv. The less negative value due to agglomeration between the particles and it is found that core particles were of spherical shapes with size variation from 40.35 to 90.47 nm average particle size was found around 80.51. Seemingly, plant-based bio-fabricated silver nanoparticle of *Russelia equisetiformis* flowers have anticancer activity in wistar rat

Keyword--Russelia equisetiformis flowers, nanoparticles, mammary tumor, progesterone

Introduction

India ranks third among the countries in terms of most cancers. According to a report of the National Cancer Registry Program, 300,000 people suffer from cancer every year in India. The incidence of breast cancer in the world is around 47.8(age-standardised rate /1lakh) [1]The main cause of cancer among people in India is tobacco, cigarette, alcohol consumption and unhealthy lifestyle which causes obesity, hormonal imbalance and change of body homeostasis For thousands of years, efforts are being made to prevent cancer with drugs and have been found to a great extent, but Cancer drugs still have flaws, Like this medicine has many side effects and this medicine fails to prevent cancer metastatic stage. The plant-derived compounds have always been an important source of medicines for various diseases and have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo preventive effects [2] It has been demonstrated that the secondary metabolites produced by plants have anticancer effects. These effects include the capacity to kill cancer cells, inhibit the growth and development of cancer cells, and combat the development of multi-drug resistance in some types of cancer. It is believed that secondary metabolites found in plants can be beneficial in the process of medication development.[3]

Humans have utilized herbs to heal various ailments for thousands of years. Investigators are mainly interested in developing anticancer medicines from secondary metabolites of the plant. Flavonoid, bioflavonoids, polyphenols, tannins anthraquinones, bi & triterpenoids, alkaloids, quinones, and other secondary metabolites found in plants play an important role in cancer prevention.[4] Plant cells make many different chemicals, mostly secondary metabolites, that help them fight bacteria, fungi, cancer, and other diseases. Sources of natural bioactive molecules that stop disease-causing pathogens in both plants and people are secondary metabolites with antibacterial, antifungal, antioxidant, and anticancer effects. Also, each plant family has its own unique mix of secondary metabolites, which means that they have different antibacterial, antifungal, antioxidant, and anticancer effects.[5] The production method of Bhasma, which incorporates metals, minerals, and animal products, plays a special role in the raw material composition in the end product. The particle size of the drug is reduced greatly as a result of multiple phases of processing procedures such as shodhana (which comprises roasting with the addition of herbal fluids and constant stirring) and marana (which involves bhavana (wet trituration) and the puta system of heating).

The particle size in Bhasma is 1-2, which might be described as the condition for the end product meeting all of the customary parameters under Bhasma pariksha (examination of properly prepared Bhasma). Despite the fact that Bhasmas are complicated materials, physicochemical analysis utilising modern techniques will be most appealing for standardising Bhasma medicines. This would undoubtedly contribute to increased trust in the use of such items for medication by ensuring

safety, efficacy, and batch-to-batch consistency.[6] Bottom-up and top-down methods of synthesis can be broadly classified. Bottom-up approaches involve the formation of NPs from molecules or atoms, whereas top-down procedures require the decomposition of bulk materials into smaller particles, which finally leads to NPs.

However, there are three best methods for producing various types of nanoparticles: physical methods, chemical methods, and biological (bio fabricated) methods.[7] Nanotechnology sculpts the current scenario of science and technology. The word nano refers 'small' which ranges from 10 to 100 nm in size. Silver and gold nanoparticles can be synthesized at nanoscale and have unique biological properties like antibacterial, antifungal, antiviral, antiparasitic, antiplatelet, anti-inflammatory, and anti-tumor activity. The usage of silver nanoparticles (AgNPs) in dentistry and dental implants, therapeutic abilities like wound dressings, silver impregnated catheters, ventricular drainage catheters, combating orthopaedic infections, and osteointegration will be elaborated. Gold nanoparticles in recent years have garnered large importance in bio medical applications. They are being used in diagnosis and have recently seen a surge in therapeutics.[8]

The morphology, size, shape, purity, surface chemistry, and other properties of AgNPs must all be checked during the green production process. UV-Vis spectrophotometry, Powder X-ray diffraction (XRD), microscope (TEM), Scanning electron microscope (SEM), Transmission electron Fourier Transform Infrared Spectroscopy (FTIR), Dynamic light scattering (DLS), and Zeta potential analyzer, among other instruments, were used to characterize green synthesized AgNPs. Because of the shift in hue, AgNP synthesis may be seen with the naked eye at first. The synthesis of AgNPs is usually indicated by the reaction mixture's dark brown hue. UV-visible spectrophotometry is then used to validate the production of AgNPs. In UV-visible spectrophotometry, synthesized AgNPs had a prominent peak about 400–470 nm. The absorption spectra of green synthesis AgNPs were influenced by their shape, size and morphology. [9][10] Since the 1990s, natural medicine has been used as a cancer treatment and a source of compounds that fight cancer. Green synthesis often uses Indian herbal medicine as a biological entity.

AgNPs that play a unique role in modern in vitro and in vivo cancer treatments. AgNPs are plasmonic structures that can scatter and absorb light in certain places, which can be used for imaging. Due to their unique properties, AgNPs are thought to have a lot of anticancer potential in two ways: they have anticancer properties of their own and help anticancer drugs work better and last longer. At the moment, the theragnostic approach (diagnosis and treatment) is one of the most interesting and difficult ways to treat cancer on an individual level. Like AgNPs' ability to kill bacteria, their ability to kill cancer depends on how nanoparticles get into cells. This can happen through diffusion, phagocytosis, and receptor-mediated endocytosis. The cytotoxicity of AgNPs depends on their physical and chemical properties, such as their size, shape, and surface properties, which could make cancer cells take them inside.[11] For high cytotoxicity, it is feasible that nanoparticles that are tiny enough can directly adhere to the cell surface and release silver ions that cause oxidative stress.

apoptosis and the structural and functional degradation of cellular organelles, such as protein and enzyme denaturation, mitochondrial disruption, and DNA damage, can cause cancer cells to die.[12] The synthetic drugs that are currently used to treat cancer are very toxic to healthy cells, which can lead to the growth of new cancers. In this case, anti-cancer drugs made from natural resources are very important, and latest research is dedicated to finding out more about them. Various medicinal plants to find new ways to fight cancer.

Alkaloids, flavonoid, phenols taxanes, podophyllotoxins, and are some of the phytoconstituents found to stop cancer cells from growing by messing up their DNA. or the formation of the spindle during mitosis. So, the cytotoxic potential of stigmasterol and amygdalin show anticancer activity by protecting the cells against by killing cancer cells with free radicals or by making them commit suicide (apoptosis), these drugs have a lower risk of side effects. So, more research needs to be done on how to make phytochemicals from plants and other sources that are more effective and less toxic. This may increase the risk of side effects, while plant phenols, curcuminoids, and terpenoids are less likely to cause harm.[13] [14] The purpose of this research was to describe anticancer activity of green synthesis of silver nanoparticles of *Russelia equisetiformis* in wistar rat. To our knowledge, no information exists on the biological or phytochemical properties of this species.

Material and Methods

Chemical and reagents

The 7,12-dimethylbenz[a] anthracene or DMBA, which was synthesized in the United States by Sigma-Aldrich, was obtained from the S, K Trader in Indore, and Elisa Progesterone kit for hormonal assay were ordered from Maxome life science Bangalore India. Chemicals of analytical grade were employed throughout the process. The silver nitrate was supplied by Loba Chemical Pvt. Ltd. (AgNO₃, 99.8 percent), whereas Merck was the one that supplied the ethanol (Germany). deionized and Milli-Q water was utilized during whole period of studies

Extract plant material

Russelia Equisetiformis was collected from govt nursery of Indore, Dr. Naveen Jain, botanist at Govt Holkar (Model, Autonomous) Science College, Indore (MP), verified the plants (Voucher number. 01A/Bot.Holker/2020). All *Russelia equisetiformis* flowers were cleaned with deionized water and shade-dried for two weeks. *Russelia Equisetiformis* dry flowers were blended into a fine, gritty powder. Single-phase extraction was done with 30:70 v/v water and ethanol. 30 g of powdered material was mixed with 100 ml of ethanolic solvent for 10 days, then filtered. Using a magnetic stirrer, the sample was boiled at 50 degrees Celsius for 45 minutes. *Russelia Equisetiformis* ethanolic extract (REEE) was chilled and collected dry. This filtrate was used to produce silver particles as reducing agents

Screening of phytochemicals

Preliminary phytochemical study of *Russelia Equisetiformis* flower ethanol extract was performed to determine the presence of lipids, volatile oils, triterpenoids, tannins, alkaloids, phenols, flavonoids, anthraquinones, tannins, and saponin. [15][16]

Green synthesis of silver nanoparticles

The plant extract and the 0.2 M Silver Nitrite (AgNO_3) solution were combined in a round-bottom flask that was equipped with a cooling condenser and magnetic stir bar; the reaction mixture was formed. At 90-95 °C, the mixture was left to mix for three hours before being cooled to ambient temperature (immediate colour change was observed from light brown to dark brown, and thereafter no further colour change was observed even after 1 hours). Centrifugation took place two hours after the mixture had been allowed to cool. At a speed of 8000 rpm at a temperature of room temperature, the centrifugation was carried out. Black powder (REEE-AgNPs) was produced after three washings with distilled water and drying in an oven at 70°C for a few hours.

Characterization of REEE-AgNPs

The behaviour of nanoparticles, as well as their biodistribution, safety, and effectiveness, are all heavily dependent on their physicochemical features. Because of this, characterisation of silver nanoparticles (AgNPs) is essential in order to evaluate the properties of the particles that have been generated. Analytical methods such as dynamic light scattering (DLS), Zeta potential, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDX). Dynamic Light Scattering (DLS) measures the hydrodynamic diameter of nanoparticles in solution and provides information on the aggregation state of nanoparticles in solution. Zeta potential is a physical property that is determined by the nanoparticles' net surface charge. This property is determined when the nanoparticles in a solution repel each other's charges due to a Coulomb explosion that occurred between the charges of the nanoparticles, which resulted in the particles not having any tendency to agglomerate together. When the values of the zeta potential range from higher than +30 mV to lower than -30 mV, we can determine whether or not the NPs meet the criteria for stability.[18][19] The scanning electron microscope (SEM) is one of the most often used tools for analysing nanomaterials and nanostructures. The signals produced by electron-sample interactions provide information about the sample such as surface morphology (texture) and chemical composition. *Russelia equisetiformis* nanoparticles were suspended in deionized water and used for SEM analysis by placing a drop of suspension into a clean electric stub and allowing the water to completely evaporate. An elemental analysis and quantitative compositional information are also provided by an Energy Dispersive X-Ray Analyzer (EDX or EDA).[20]

Animals

All in vivo experimental animals were donated by the Acropolis Institute of Pharmaceutical Education and Research in Indore (1627/PO/Re/S/12/CPCSEA). The Institutional Animal Ethical Committee (IAEC no-AIPER/IAEC/2021/002) approved the procedure. All animal studies were carried out in compliance with CPCSEA guidelines and regulations. Rats were given food and water on an ad libitum basis (standard pellet). The experimental work with the rats began after a seven-day acclimation period. Each cage accommodated two rats, who were allocated into two groups using a randomized distribution mechanism. The treatment group received therapy (REEE and standard), whereas the control group received no therapy. The mean room temperature of the animal housing was maintained at (24°C 2°C) for the rats in the experiment utilizing a 12-hour light/dark cycle.

Acute toxicity studies

In the present study, the acute toxicity was carried out by the “Fixed Dose Method” of the OECD 423 guideline. The rats were orally administered with REEE extract and REEE-AgNPs with three-three albino Wistar rats were chosen for each phase with oral administration of the doses at 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg Depending on the mortality rate, two to four stages may be required to make a decision on the acute toxicity of the test drug, and/or the animals' morbidity condition. [21]

S.No	Group	Group	Dose, drugs, and Schedule
1	Group-I	Control Group	Only 1mL of saline was given. 0 th to 101 days
2	Group II	Negative control	All rats Only received DMBA (S.C 50 mg/kg) at 0 th days and were induced by subcutaneous administration in 1 ml sunflower oil
3	Group III	Only Extract (REEE)	All Rats treated with DMBA (similar to group-II) +extract (REEE) 100 mg/kg given orally at 51 days from 0 th day
4	Group IV	REEE - AgNPs Low dose	All Rats treated with DMBA (similar to group-II) +nanoparticle (REEE-AgNPs) 50 mg/kg/day given orally at 51 days from 0 th day
5	Group V	REEE - AgNPs High dose	All Rats treated with DMBA (similar to group-II) +nanoparticle (REEE-AgNPs) 100 mg/kg given orally at 51 days from the 0 th day
6	Group VI	Standard (Positive control)	All Rats treated with DMBA (similar to group-II) + Tamoxifen(20mg/kg)

Experimental design and procedure

For this study, healthy female Wistar rats aged 56 to 60 days were taken and the average weight of the rats was 170 grams. as per the study design show(table no 01), rats were randomized and divided into 6 groups Shown in table no 1. DMBA is given to all groups (Group II to VI) except Vehicle Control Group (Group-I) and it is considered this group as the 0th day. The rats were palpated twice a week for the identification of breast cancers. During the whole experiment, all groups of rats weighed 0th days intervals. after that, drug dosing is started when the tumor size reaches 0.5 cm. All rats were given the drug dose as per the study design from day 56th to 100 days. On day 101, all rats that developed tumors during the induction period are carefully and safely sacrificed by diethyl ether in the diestrus phase of the estrous cycle. At the end of the experimental period, the rats were weighed. The weight of the animal & tumor volume was calculated using the standard formula: $\text{Width}^2 \times \text{length} \times 0.5$. After the blood collection, the animals were sacrificed and the entire liver and other tissues was perfused immediately with ice-cold 0.9% sodium chloride, and then carefully removed, trimmed free of extraneous tissue finally the mammary tumors were excised out and the following parameters were studied.[22]

- Tumor weight. (Initial Vs final weight)
- Tumor incidence (% of animals that develop at least one tumor)
- Tumor burden (average number of tumors per animal)
- Tumor volume

Biochemical Assay

SGOT, SGPT, ALP and Bilirubin, performed by standard kits of Span Diagnostics Ltd. Centrifugation at 3000 rpm at 4°C for ten minutes was used to separate the serum. biochemical markers, including SGPT, SGOT, ALP and bilirubin, were measured in the serum that was obtained by centrifuging the blood that had been taken. The enzyme known as serum glutamate oxaloacetate transaminase (SGOT) is responsible for the transamination that leads to the formation of oxaloacetate and L-glutamate from L-aspartate and -ketoglutarate. The generated oxaloacetate is associated with 2,4-dinitrophenylhydrazine (DNPH), which results in the formation of a hydrazone. A hydrazone is a brown-coloured complex in an alkaline solution that is measurable using calorimetry. Transamination of L-Alanine and Ketoglutarate to L-Glutamate is carried out by SGPT (serum glutathione peroxidase). In an alkaline media, the resulting hydrazone, a brown complex that can be detected calorimetrically, is generated when pyruvate is combined with 2,4-dinitrophenylhydrazine. AST (GOT/GPT) activity in IU/L = $[(\text{Absorbance of the test} - \text{Absorbance of control}) / (\text{Absorbance of standard} - \text{Absorbance of blank})] \times \text{concentration of the standard}$ [23]. At pH 10.0, serum alkaline phosphatase (ALP) transforms phenyl phosphate to inorganic phosphate and phenol. In an alkaline media, phenol interacts with 4-aminoantipyrine in the presence of the oxidizing agent potassium ferricyanide to generate an orange-red colored complex that can be detected spectroscopically. The intensity of the hue is related to the enzyme activity.

$$\text{ALP} = [(\text{O.D. Test} - \text{O.D. Control}) / (\text{O.D. Standard} - \text{O.D. Blank})] \times 10$$

Total Bilirubin in serum was determined using Jandrossik and Grof method by coupling with diazotized sulfanilic acid after addition of caffeine sodium benzoate and sodium acetate. A blue azobilirubin is formed in alkaline Fehling solution II, which is measured photometrically.[24]

Hormone assay

The ELISA technique was used to evaluate progesterone hormone level in wistar rat. CALBIOTECH Progesterone Elisa Kit use to performed female wistar rat progesterone level (LOT NO. PGS6303). The standard progesterone hormone range was calibrated before collecting 25 L of serum samples on the microwell plate. First, 50 L of progesterone enzyme reagent was poured into each microwell. After carefully mixing the microwell plate for 20–30 seconds, 50 L progesterone biotin reagent was applied to each well. The microwell plate was gently stirred for another 20–30 seconds before being covered and incubated at room temperature for 60 minutes. The contents of the microwell plate were then discarded and rinsed three times with 350 L of wash buffer before being blotted. Each well received 100 L of substrate solution and was incubated at room temperature for 20 minutes. Finally, 50 L of stop solution was added to each well and gently mixed. The absorbance was measured in ng/mL using a Merck ELISA reader at 450 nm (with a reference wavelength of 620–630 nm).[25]
[26]

Histopathological study

Breast tissues were fixed in 10% formalin for histological examinations.

Statistical Analysis

Using of stat3.2 software, Data were expressed as mean \pm Standard Deviation (S. D). All of the data were compared using one-way ANOVA with Dunnett's post-test. If ≤ 0.05 , a variance was considered statistically significant.

Result

Phytochemical analysis

A phytochemical group test was done for tannins (using FeCl_3 test, $\text{K}_2\text{Cr}_2\text{O}_7$ test, and lead acetate test), glycosides (using Legal's test, Keller-test, Killiani's and Borntrager's test), and vitamins (using Legal's test, Keller-test, Killiani's and Borntrager's test). Saponins (using the foam test), alkaloids (using the Mayer's test, Dragendorff's test, Wagner's test, and Wagner's test). phytosterols (using Liebermann-test Burchard's and the Salkowski reaction), sugar reduction (using Fehling's and Benedict's tests), flavonoid reduction (using Shinoda and Benedict's tests), zinc hydrochloride reduction test), as well as proteins and amino acids (using the biuret and ninhydrin tests). polyphenols (as tested) (FeCl_3 test). *Russelia equisetiformis* ethanolic extract discovered the Polyphenol saponins, glycosides, triterpenes, tannins, and flavonoids are present. These Chemicals may have a key role in the reduction or conversion of silver ions. nanoparticles.

Characterization of REEE-AgNPs

Dynamic light scattering by Delsa™ Nano Common Beckman Coulter machine showing (fig-01) intensity distribution and auto correlation function DLS is a non-destructive method and provides information about the average size of the nanoparticles dispersed in a liquid medium. The synthesis of the nanoparticles in this study was carried in a liquid medium, which was directly subjected to DLS in order to determine the size of the nanoparticles. It is observed that the size distribution of AgNPs ranges from 50 to 950 nm. The average size of AgNPs synthesized from 283.6 nm and Polydispersity Index is 0.483 (With various measurement condition like viscosity 0.88 cps and temperature 24.4-degree c). This wide range of size is most probably because of tendency of nanoparticles to form agglomerates over time. The zeta potential (fig-02) of the synthesized REEE-AgNPs was determined in water as dispersant. The zeta potential was found to be -12.34. mv.

The less negative value due to agglomeration between the particles and thereby increase instability of the formulation. The morphological study was performed by SEM and obtained photomicrographs are presented in Figures 3a and 3b. SEM images of formulated silver nanoparticles of RE plant extract are shown at different magnifications. It can be seen that core particles were of spherical shapes (Fig.3a) with size variation from 40.35 to 90.47 nm average particle size was found around 80.51 nm moreover few cubic and rod like nanostructure also found at 10x magnification (Fig. 3b) using an advanced software named "IMAGEJ". The big size variation could be due to the agglomeration of individual nanoparticles or subunits into larger particles. The EDX data shows energy dispersion. Fig4 REEEE-AgNPs are surface bio fabricated nanoparticles consisting of C, AgO, Cl, and O. The silver is reported to be 60% with 35% of carbon, confirming that the nanoparticles are free of contaminants.

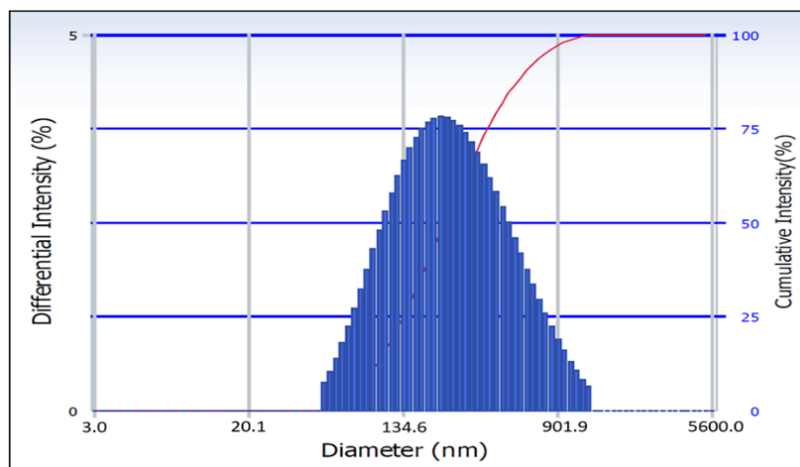


Fig 1. DLS of REEE-AgNPs

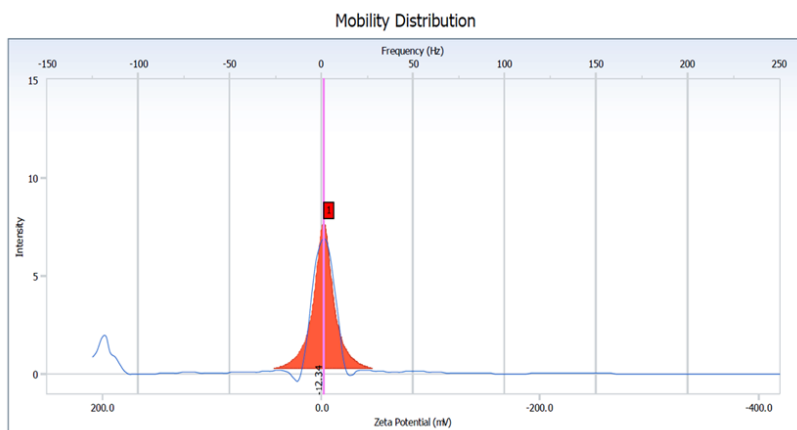


Fig 2. Zeta potential of REEE-AgNPs

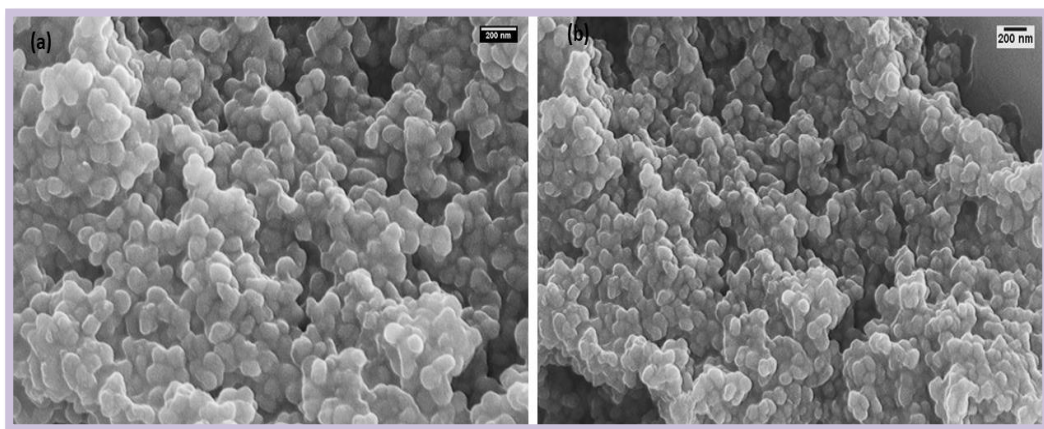


Fig 3. (a &b) SEM of REEE-AgNPs

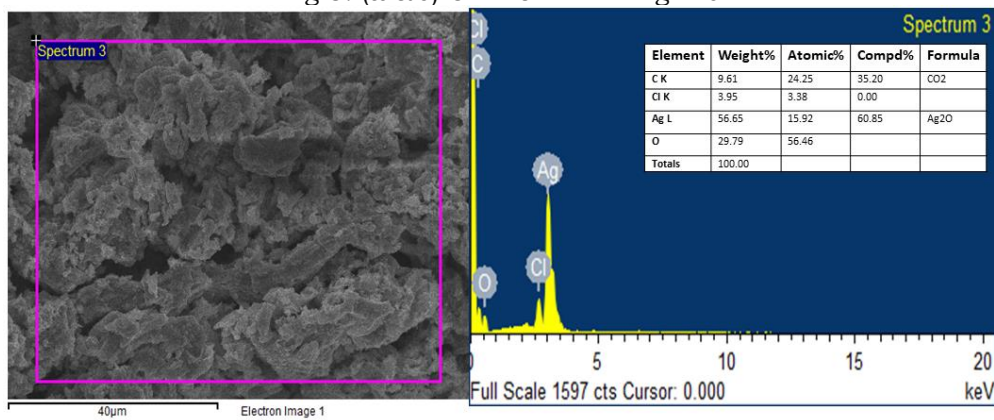


Fig 4. EDX of REEE-AgNPs

Results of acute toxicity studies

Toxicity testing was carried out on both of them REEE and REEE AgNPs formulations in accordance with OECD criteria. Toxicological tests REEE and REEE AgNPs were supplied orally to the rats at doses ranging from 5 mg/kg to 300 mg/kg to 2,000 mg/kg. The rats showed no clinical symptoms of toxicity or mortality after being given the tests REEE and REEE AgNPs. All of the animals gained weight and showed no signs of behavioural alteration, indicating that the administration of both (REEE) and REEE AgNPs) extracts had a minor effect on the animals' growth. LD50 values were determined to be larger than 2000 mg/kg in all of the dosages tested and whole toxicity studies found that there is no deaths or clinical symptoms of toxicity at any of the doses examined (Table-02). When given to rats at a dosage of 2000 mg/kg, it has been shown that both the extract REEE and REEE-AgNPs formulations are not deadly. As a result, a 1/10th dose of 200 mg/kg was chosen as the high dose for both REEE and REEE AgNPs, and a 100 mg/kg dose is being evaluated for additional biological investigations. The results of the observations are listed in the following table:

S.no	Test Sample	5 mg/Kg	50 mg/Kg	300 mg/Kg	2000 mg/Kg
1	REEE	None	None	None	None
2	REEE-AgNPs	None	None	None	None

Bodyweight and tumor parameter

All the animals in all the groups were alive during the experiment. Bodyweight was assessed throughout the study and it was found that there was no significant change (fig-05) in body weight at early stages in all animals (table -03). there is a statistically significant difference ($P < 0.001$) found between all groups except group 4 and group 3. Volumr of tumor is equally importatn to diffretial tumor size in diffent group two group(G1 & G6) was remove from statical analysisi Because the tumor did not develop in the group. All the rest of the group was less but we did negative control group G2(Table 4) and in which the tumor incidence was hundred percent which graudal decess with increase of dose but only G5((REEE -AgNPs-200mg) show hight signinfcate anticancer activity.Although G4 was shown anticancer activity but it is much less in comparison to G5.

S.no	Groups	Initial body weight (gm)	Final body weight (gm)
1	G1 -Control group	178.3±2.3	246.3±8.2
2	G2 - (Negative control) DMBA	180.8±5.8	148.0±1.6 ^a
3	G3-Only Extract (REEE) 100mg	173.8±2.0	178.8±2.1
4	G4 - REEE -AgNPs(100mg)	176.3±6.3	180.3±0.9
5	G5 -REEE -AgNPs(200mg)	173.9±2.7	195.1±3.7 ^a

6	G6 - (Standard) tamoxifen	171.9±1.71	271.7±7.3 ^a
Value is expressed as mean ±SD for all six animals in all groups Superscript “a” is donated as the significant difference (P <0.001)			

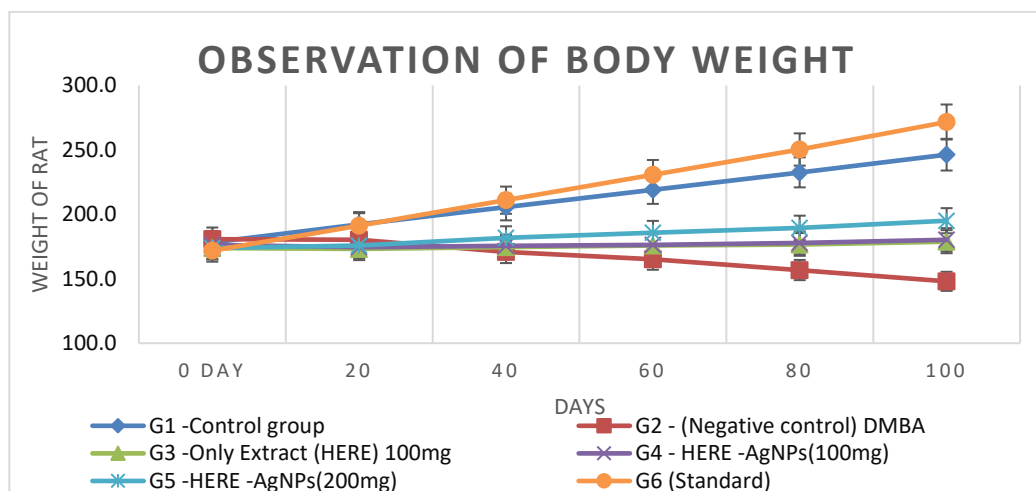


Fig 5. Observation of Bodyweight

S.no	Group	Tumor Incidence	Total No of Tumor	Tumor Volume (mm ³)
1	G1 (Control Group)	None	None	-
2	G2(Negative control) DMBA)	100%	6/6	4673.7±404.3
3	G3(Only Extract REEE-100mg)	83.3	5/6	3895.6±590.3
4	G4 (REEE-AgNPs-100mg)	66.6	4/6	3204.8±709.3 _b
5	G5 (REEE -AgNPs-200mg)	50	3/6	1589.5±602.8 _a
6	G6 (Tamoxifen -Standard)	0%	0/6	-

Value is expressed as mean ±SD for all six animals in four groups Superscript “a” is donated as the significant (P <0.001) and b is(P< 0.002)

Biochemical Assay by Serum

Table 5
Biochemical Assay by Serum

Group	SGOT	SGPT	ALP	Bilirubin
	Mean ±SD	Mean± SD	Mean± SD	Mean± SD
G1 (Control Group)	50.14 ± 1.60	17.51±2.04	63.45±4.36	0.087±0.01
G2(Negative	127.81	106.77±4.03	216.75±4.05	1.645±0.14

control- DMBA)	±4.71			
G3(Only Extract REEE-100mg)	108.83 ±5.85a	82.33±4.59 b	143.01±3.99 a	0.720±0.08 b
G4 (REEE-AgNPs-100mg)	110.77 ±3.45a	75.12±4.42 b	140.40±4.03 a	0.685±0.08 a
G5 (REEE -AgNPs-200mg)	96.33 ±3.33 a	67.15±2.61 a	132.77±2.93 a	0.598±0.11 a
G6 (Tamoxifen -Standard)	65.78 ±3.31a	31.13±2.20 a	84.02±3.60 a	0.173±0.01 a

Value is expressed as mean ±SD for all six animals in four groups Superscript “a” is donated as the significant (P <0.001) and b is(P< 0.002)

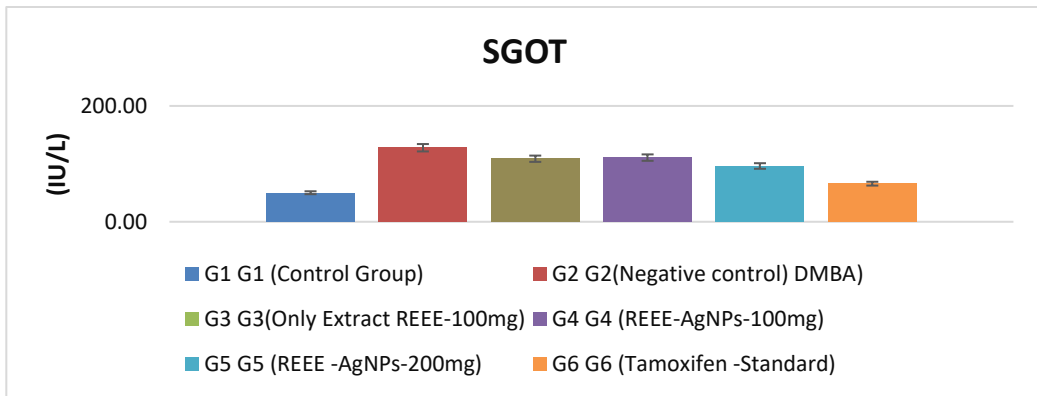


Fig 6. Biochemical Assay by Serum (SGOT)

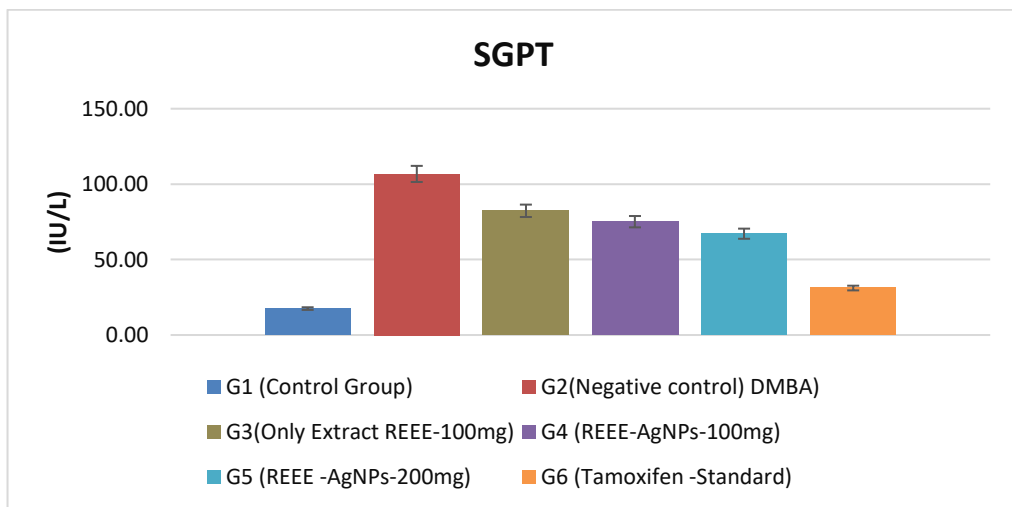


Fig 7. Biochemical Assay by Serum (SGPT)

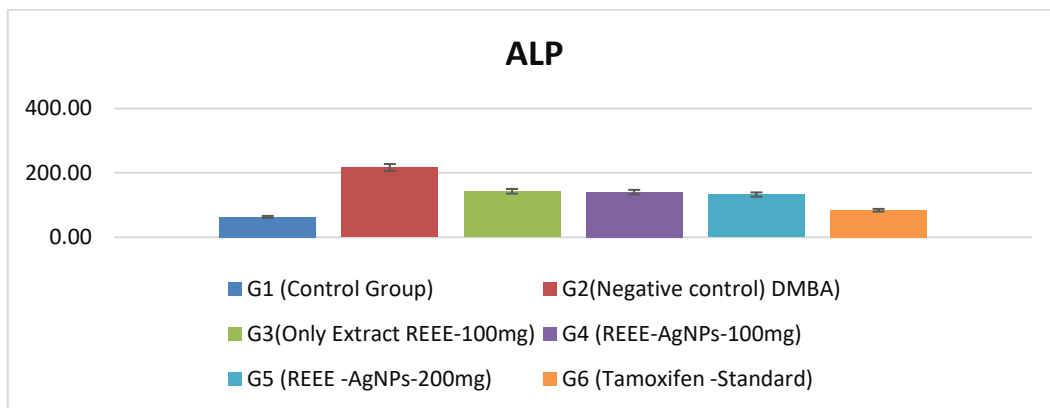


Fig 8. Biochemical Assay by Serum (ALP)

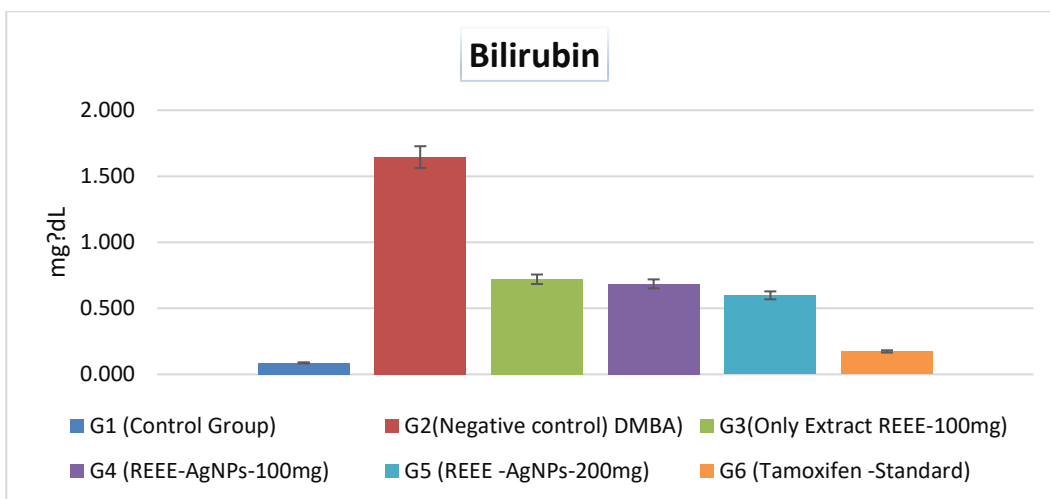


Fig 9. Biochemical Assay by Serum (Bilirubin)

Hormone assay

Progesterone level (Fig 10) was analysed by ELISA method. The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference ($P = 0.895$).

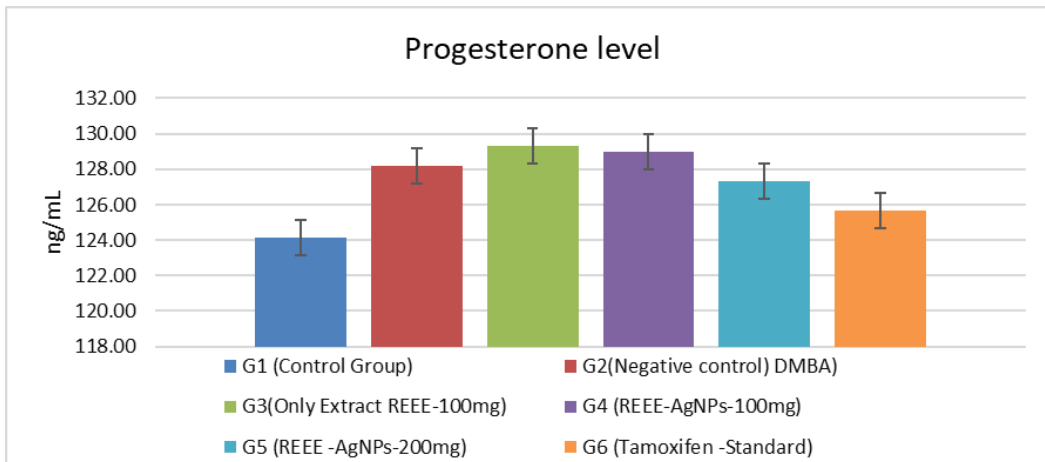


Fig 10. Progesterone level

Histopathological study

The Histopathology of breast tissue show in Fig 11.(A) myoepithelial cells and Basement membrane with less occupied area of stromal cell (400X) (B) Intra-ductal proliferation Tumor cells and stroma area and periductal stromal fibrosis and fatty tissue (100X) (C)Tubular adenoma showing proliferating ductal and alveolar structures arranged in clusters(100x) (D)circle show the cystic changes in alveoli field showing constricted and distorted alveoli with distinct myoepithelial cells surrounded by thick layer of connective tissue (100x) (E)showing tumor cell indicating low grade hyperplasia(100x) F) showing epithelial cells of acini round to oval nucleoli and deposition of lipid droplets (400X).

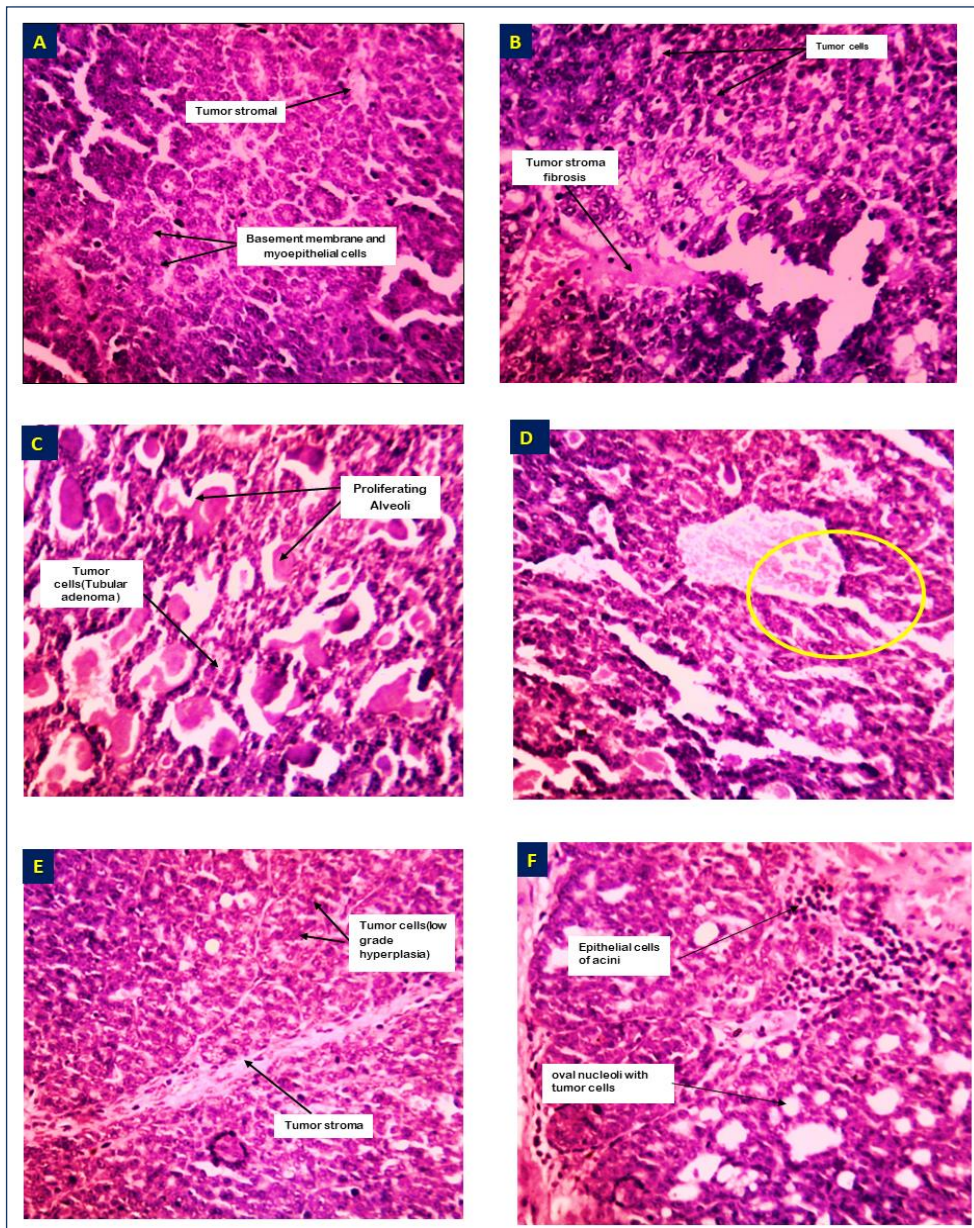


Fig 11. Histopathology of breast tissue

Discussion

Breast cancer is the most common type of cancer in women. Despite the numerous breast cancer treatment options available, they have limitations and are often rejected by cancer. Nanotechnology aids in the diagnosis and treatment of cancer, particularly breast cancer. The origin of breast cancer is complicated by lifelong exposure to endogenous and exogenous influences. Female developing bodies may necessitate different time and dose. Transforming, dysregulating

apoptosis, proliferating, migrating, angiogenesis, and metastasizing are all ways for cancer cells to avoid apoptosis. Plant-based anticancer medicines have shown promise in clinical trials, and doctors are employing them. They fortify body systems that have been stressed by chemical toxicity or trauma. Natural therapies, such as plant-derived drugs, may help to reduce the dangers of chemotherapy. Plants and other natural products have long been used as anti-cancer treatment options. Natural remedies are the basis for vinblastine, vincristine, paclitaxel, and camptothecin. Flavonoids, tannins, curcumin, resveratrol, and gallacatechins are examples of polyphenols. Resveratrol can be found in nuts, grapes, and wine.

Gallacatechins are found in tea. Polyphenols, which are natural antioxidants, may boost health and reduce the risk of cancer. The cytotoxicity of polyphenols on cancer cells and their antioxidant properties have been proven. Flavonoids are made up of polyphenolic compounds that are part of a large family of bioactive phytochemicals known as flavonoids, of which there are 10,000 known structures. [27] The extract includes all sorts of phytochemicals that are responsible for anticancer and antioxidant activities, such as delay petrol election migraine, according to the current study. The current study found polyphenol saponins, glycosides, triterpenes, tannins, and flavonoids in the ethanolic extract of *Russelia equisetiformis*. For these reasons, *Russelia equisetiformis* was employed as a natural reducing agent in the green production of silver-based nano particles. As a solvent for natural chemicals used in both food and natural medicine, ethanol is safe for human consumption. With good findings, absolute ethanol and aqueous ethanol have been used to extract phenolic compound antioxidant compounds from natural sources. Nanoparticles improve the compatibility and bioavailability of natural compounds in chronic disease treatment, including cancer.

AgNPs are popular in disease management because they disturb the mitochondrial respiratory chain. AgNPs affect mitochondrial function by generating ROS and inhibiting ATP synthesis, causing DNA damage. Characterized the nanoparticles in 0.2 M Silver Nitrate (AgNO₃) concentration and discovered that the nanoparticles aggregated in place but were 80.5 nm in size. The presence of a silver on the surface of the nanoparticles is demonstrated by EDX measurement, and the silver is present throughout the formulation with herbal extract. Outcome of acute toxicity reveal that there is no toxicity found in both *Russelia Equisetiformis* ethanolic extract (REEE) and silver nanoparticles of *Russelia Equisetiformis* ethanolic extract (AgNPs-REEE). Because cancer cells require more energy, you may burn more calories at rest. After induction, groups G1, G5, and G6 have shown an increase in final weight, where as the G3 group did not find weight gain as compared to the negative control group. It's also found that there is less tumour incidence in the treatment groups, especially tamoxifen (Standard) group, than in the REEE-AgNPs (200mg). REEE-AgNPs, both dosed (high and low), and only the REEE dose, have a considerable therapeutic potential to reduce organ damage in DMBA-induced tumours in rats.

The active ingredient of the *Russelia Equisetiformis* ethanolic extract significantly reduces the increased levels of SGPT, SGOT, ALP, and bilirubin in the DMBA-induced mammary tumour model. The anticancer effect of *Russelia Equisetiformis*

ethanolic flower extract may be connected to the free radical-scavenging and antioxidant activities of several phenolic or flavonoid chemicals found in the extract. It is reasonable to infer that the silver nanoparticles of *Russelia Equisetiformis* ethanolic floral extract are more effective than the *Russelia Equisetiformis* extract. One of the most essential aspects in the development of breast cancer is the binding of receptors on the mammary gland epithelial cells by the steroid hormones oestrogen and progesterone, which promote the proliferation of neoplastic cells. We didn't find any significant changes in the levels of the progesterone hormone during the diestrous phase of the rat's estrous cycle, but these levels may change during other parts of the cycle. The difference in the degree of severity between the *Russelia Equisetiformis* treated group and the DMBA treated group is evidence that the anti-proliferative nature of *Russelia Equisetiformis* extract and silver nanoparticles of *Russelia Equisetiformis* extract is confirmed by the histopathological study as well. As a consequence of the desmoplastic response, the existence of thick fibrous to collagen tissue reaction in the tumour mass provides evidence for the protective and anti-proliferative capabilities of the *Russelia Equisetiformis* ethanolic floral extract containing silver nanoparticles.

Conclusion

The findings of the whole research led the researchers to conclude that the plant extract and the nanoparticles present in it have the ability to limit the spread of tumor cells to some extent. This also demonstrates that the extract of *Russelia Equisetiformis* and silver nanoparticles derived from the flowers extract of *Russelia Equisetiformis* cannot be used as a kind of hormone treatment in the prevention of ductal adenocarcinoma. In a comparison study, we found that silver nanoparticles of *Russelia Equisetiformis* ethanolic extract are more effective at fighting cancer than the extract itself.

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